

No Effect of Pre-exercise Meal on Substrate Metabolism and Time Trial Performance During Intense Endurance Exercise

*David Paul, Kevin A. Jacobs, Raymond J. Geor,
and Kenneth W. Hinchcliff*

To determine the effect of macronutrient composition of pre-exercise meals on exercise metabolism and performance, 8 trained men exercised for 30 min above lactate threshold (30LT), followed by a 20-km time trial (TT). Approximately 3.5 h before exercise, subjects consumed a carbohydrate meal (C; 3 g carbohydrate/kg), an isoenergetic fat meal (F; 1.3 g fat/kg), or a placebo meal (P; no energy content) on 3 separate occasions in randomized order. Treatments had no effect on carbohydrate oxidation during exercise, but C decreased whole-body fat oxidation during the last 5 min of 30LT and TT, respectively (3.2 ± 1.6 and $4.8 \pm 2.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < .05$) when compared to F (13.3 ± 1.6 and $16.5 \pm 2.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and P (15.9 ± 2.7 and $17.0 \pm 3.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Glucose rate of appearance (R_a) and disappearance (R_d), and muscle glycogen utilization were not significantly different among treatments during exercise. TT performances were similar for C, F, and P (32.7 ± 0.5 vs. 33.1 ± 1.1 and 33.0 ± 0.8 min, $p > .05$). We conclude that the consumption of a pre-exercise meal has minor effects on fat oxidation during high-intensity exercise, and no effect on carbohydrate oxidation or TT performance.

Key Words: fat oxidation, carbohydrate oxidation, stable isotopes, glucose kinetics

As exercise progresses beyond a moderate intensity (60–70% $\text{VO}_{2\text{max}}$), the rate of carbohydrate oxidation increases and the rate of fat oxidation decreases (1, 2), while plasma free fatty acid (FFA) concentrations decline (2, 3). Romijn et al. (3) explained the decline in FFA concentrations by demonstrating that the rate of appearance (R_a) of FFA does not increase during the transition from rest to exercise at 85% of maximal oxygen consumption ($\text{VO}_{2\text{max}}$), despite a 4–5 fold increase in fat oxidation. In the same investigation, it was reported that increasing the availability of FFA increased fat oxidation by 27%. Several investigations have also found that increasing FFA availability prior to high intensity exercise (>75% $\text{VO}_{2\text{max}}$) increased the rate of fat oxidation, resulting in a “sparing” of endogenous carbohydrate (3–6).

D. Paul is with the United States Department of Agriculture, Building 308, Center Rd., Beltsville, MD 20770. K.A. Jacobs is with the School of Physical Activity and Educational Services at The Ohio State University, Columbus, OH 43210. R.J. Geor and K.W. Hinchcliff are with the Department of Veterinary Clinical Sciences at The Ohio State University.

Although the concentrations of circulating FFA and glucose can be readily manipulated by altering pre-exercise meal macronutrient composition or by fasting, it is uncertain whether these changes alter substrate metabolism during exercise. All of the aforementioned reports demonstrating a carbohydrate-sparing effect due to the increased availability of plasma FFA used Intralipid and/or a fat meal combined with heparin injections (3–6). In addition, it is unknown if these changes in substrate metabolism will result in an improvement in exercise performance.

The mechanisms of fatigue during high intensity endurance exercise of a relatively short duration (≈ 1 h) are not well understood, but a reduction in the use of endogenous carbohydrate may be of great importance to endurance sports such as cycling, since fatigue during this type of exercise may be due to the depletion of endogenous carbohydrate stores (7, 8) and/or inability to maintain a high rate of carbohydrate oxidation (9). On the contrary, other authors have suggested that carbohydrate depletion is not a mechanism of fatigue at high intensities (10). Thus, macronutrient composition of a pre-exercise meal (or no meal at all) may have no effect on the performance of endurance athletes entering a competition, assuming their carbohydrate stores are not compromised at the beginning of exercise. This is of particular importance to athletes who are uncomfortable consuming meals prior to competition due to the potential for gastro-intestinal distress and/or pre-competition nervousness.

Studies investigating the efficacy of pre-exercise meals at exercise intensities, where the availability of FFA may be compromised ($>75\% \text{VO}_{2\text{max}}$), are few in number and have found mixed results (11–13). Furthermore, no investigations have compared the effects of carbohydrate and fat meals, and a fast (no meal) in the same subjects. Therefore, the primary purpose of the present study was to compare the effects of a fat- and carbohydrate-rich meal 3–4 h before exercise compared to fasting on whole-body fat and carbohydrate oxidation, muscle glycogen utilization, and rates of appearance (R_a) and disappearance (R_d) of blood glucose during 30 min of cycling above lactate threshold in carbohydrate-replete subjects. A secondary purpose was to determine if there is an effect of pre-exercise meal on 20-km time trial performance that followed the 30 min of exercise.

Methods

Subjects

Eight endurance-trained men (Table 1) participated in the study after providing written informed consent. The subjects were amateur competitive cyclists and triathletes who were in training for competition or in the competitive phase of their season.

Pre-experimental Protocol

$\text{VO}_{2\text{max}}$ was determined by an incremental cycle ergometer test to exhaustion (Velodyne cycle ergometer, Frontline Technologies, Irvine, CA, USA). Beginning with an initial workload of 75 or 100 W (depending on the body mass of the subject), the workload was increased by 50 W every 2 min until exhaustion. The $\text{VO}_{2\text{max}}$ was defined as the attainment of at least two of the three following criteria: (a) an increase of $\leq 150 \text{ ml VO}_2$ with an increasing workload, (b) respiratory exchange ratio (RER) ≥ 1.10 , and (c) heart rate within 10 beats of age-predicted maximum. Within 1 week of the incremental exercise test, subjects performed a lactate threshold

Table 1 Physical Characteristics of the Subjects (N = 8)

Variable	Mean	SE
Body fat (%)	13.4	3.4
Body weight (kg)	73.4	7.4
VO _{2max} (ml · kg ⁻¹ · min ⁻¹)	64.1	7.9
(L/min)	4.7	0.6
Peak power (W)	366	30.6
Lactate threshold		
Power (W)	225	34
VO ₂ (L/min)	3.3	0.6
%VO _{2max}	69.3	3.4
Above lactate threshold		
Power (W)	246	32
VO ₂ (L/min)	3.7	0.6
%VO _{2max}	76.8	3.7

(LT) test modified from a technique described previously (14). Subjects began exercise at a power output corresponding to approximately 30–40% VO_{2max} and, every 5 min, the power output was increased by 25 W until the subject was exhausted. Blood samples were drawn during the last min of each stage via a catheter that was placed in a forearm vein. *Lactate threshold* was defined as the workload that resulted in an increase in plasma lactate > 1 mM above the mean lactate observed during the initial two stages of the test.

Body density was assessed by hydrostatic weighing, and residual volume was estimated by the nitrogen dilution method (15). Body composition was determined using the equation of Siri (16).

Experimental Protocol

On the evening before each experimental testing session, subjects exercised for 30 min at approximately 60% VO_{2max}. After exercise, they were fed a high-carbohydrate meal in the laboratory (2.6 g carbohydrate, 0.03 g fat, 0.16 g protein/kg body weight). The next morning (12 h after the previous meal), subjects were provided with a standardized high-carbohydrate breakfast consisting of 2 g carbohydrate, 0.03 g fat, and 0.16 g protein/kg body weight. The purpose of these trials and feedings was to ensure that all subjects were well rested and began the experimental protocol with replete stores of liver and muscle glycogen.

Approximately 3.5 h before exercise (6 h after breakfast), subjects were provided with one of the pre-exercise meals in liquid form. The carbohydrate-rich meal (C) contained 3 g carbohydrate/kg body weight, and consisted of a sports-drink, fat-free non-dairy creamer powder, artificially-sweetened lemon-flavored powder, and water. The isoenergetic fat-rich meal (F; 1.3 g fat/kg body weight) consisted of

heavy whipping cream, water, and an artificially sweetened, lemon-flavored powder. The placebo beverage (P) contained no calories and consisted of calorie-free gelatin powder, lemon-flavored powder, and water. The flavor and texture of the meals were designed to be indistinguishable from each other and were provided in containers that prevented the subjects from seeing the contents. As suggested by Clark et al. (17), we took great care to ensure that treatments were indistinguishable from each other. The meals were partitioned into three separate portions that were consumed at 15-min intervals (one-half of the meal initially, and the remainder in two 15-min intervals). The order of the double-blind trials was randomly assigned and crossed-over, with 5–10 days separating each trial. The timing of the fat meal (and corresponding carbohydrate meal and placebo beverage) was chosen because of the time it takes for exogenous FFA to enter circulation (18).

Two and one-half hours before exercise, catheters were placed in a forearm vein of one arm for blood sampling and in a vein of the contralateral arm for isotope infusion. The forearm designated for blood sampling was wrapped with a heating pad to obtain arterialized venous blood samples (19). Catheter patency was maintained in the blood-sampling catheter by periodic flushing with saline and heparin. After a priming dose of $17.6 \mu\text{mol/kg}$, 99% enriched $[6,6\text{-}^2\text{H}]\text{glucose}$ (Cambridge Isotopes, Cambridge, MA, USA) was continuously infused at a rate of $0.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 2 h of rest. The tracer infusion rate was tripled during exercise. Approximately 1 h after the beginning of tracer infusion, a muscle biopsy was taken from the vastus lateralis muscle and immediately frozen in liquid nitrogen for the subsequent analysis of muscle glycogen concentration.

Each experimental session began with subjects exercising at 40% $\text{VO}_{2\text{max}}$. The power output was then increased by 10% $\text{VO}_{2\text{max}}$ after 2 min and 5 min of cycling. After 10 min of cycling (warm-up), the power output was increased to approximately 25 W above LT, and this load was sustained for an additional 30 min (30LT). Workloads were set by LT in order to reduce variability in glucose R_a and muscle glycogen utilization associated with setting workloads by $\text{VO}_{2\text{max}}$ (20). Second, pilot work in our laboratory found that setting workloads at approximately 25 W above LT resulted in high power outputs that could be sustained for 30 min without resulting in premature exhaustion (stopping prior to the end of the 30 min steady state exercise period; 30LT). The subjects then rested for approximately 15 min prior to completing a 20-km time trial (TT). During this rest period, the tracer infusion was stopped, infusion tubing was removed, and a second muscle biopsy was taken. At least one "acclimation" session was performed on each subject prior to beginning the study. (No blood was drawn or biopsies taken.)

The TT was performed on the same bicycle/ergometer setup as the initial exercise period. Subjects were instructed to cover 20-km in the shortest time possible. Feedback regarding the distance covered was provided, but subjects were not allowed to observe time elapsed, heart rate, or any information regarding previous performances. This approach has been shown to be a reliable and valid means to measure cycling performance (21). Subjects were given no verbal encouragement by any of the investigators.

Respiratory gas samples were collected continuously for the first and last 5 min of 30LT, and for 5 min during the beginning of the TT. Heart rate was monitored continuously during exercise by telemetry. The laboratory environment was maintained at approximately 23 °C and 60% RH, and electric fans were directed on the subjects to aid in heat dissipation.

Blood Sampling

Blood samples were collected before isotope infusion, and 30, 15, and 0 min before 30LT. During exercise, blood samples were drawn after 10, 20, and 30 min of 30LT, and immediately before and after the TT. Blood samples for the determination of plasma glucose and lactate were placed in tubes containing sodium fluoride-potassium oxalate, while samples designated for the analysis of glucose enrichment and hematocrit were placed in tubes containing EDTA. The tubes containing sodium fluoride-potassium oxalate and EDTA were kept on ice until centrifugation. Blood samples designated for the analysis of glycerol, FFA, and insulin were placed in serum separation tubes, kept at room temperature for at least 15 min, and allowed to clot. All samples were centrifuged at 3000 rpm for 15 min at 4 °C and stored at -80 °C until analysis.

Analytical Techniques

Oxygen and carbon dioxide contents of expired gases were measured with Applied Electrochemistry S-3A/II and CD-3A analyzers (AEI Technologies, Pittsburgh, PA, USA). Respiratory gas volumes were measured with a Parvomedics pneumotachometer (Hans Rudolph, Kansas City, MO, USA). Oxygen uptake and respiratory exchange ratio (RER) were calculated by a conventional software package (Consentius Technologies, Sandy, UT, USA).

Plasma glucose, plasma lactate, serum glycerol, serum triglyceride (TG; Sigma Chemical, St. Louis, MO, USA), and serum FFA (Wako Chemicals, Richmond, VA, USA) were assessed spectrophotometrically by commercially available kits. Serum insulin was measured by radioimmunoassay (ICN, Costa Mesa, CA, USA). Muscle glycogen concentration was assessed by acid hydrolysis of glycogen residues to glucose, followed by a fluorometric analysis of glucose (22).

Determination of Glucose Enrichment

Plasma samples were deproteinized and derivatized using an adapted technique (23) prior to analysis. Plasma samples were deproteinized by the addition of 0.86 ml of ice cold 0.3 N ZnSO_4 and 0.86 ml of ice cold 0.3 N $\text{Ba}(\text{OH})_2$, vortexed, and placed in an ice bath for 20 min. Samples were then centrifuged at 3000 rpm for 20 min at 4 °C, and the resulting supernatant was lyophilized by vacuum centrifugation. The pentaacetate derivative of glucose was formed by the addition of 100 μL of a 2:1 mixture of acetic acid and pyridine, and incubated for 60 min at 60 °C. These samples were partitioned with the addition of 400 μL of dimethylchloride and 1.5 ml of distilled water, and then centrifuged at 2000 rpm for 10 min. Suction was used to remove the water phase, and the dimethylchloride phase was dried under a stream of N_2 gas. The dried pentaacetate glucose derivative was suspended in 50 μL of ethyl acetate prior to gas chromatography-mass spectrometry (GCMS) analysis, and subsequently transferred to airtight chromatography vials and glass pipettes.

Glucose isotopic enrichment was determined by GCMS (model 5989A, Hewlett-Packard, Palo Alto, CA, USA). The resulting data was processed by selected ion monitoring at mass-to-charge ratios (m/e) of 200, 201, and 202, and correction was made for the contribution of m/e 201 to the apparent enrichment of m/e 202 (24).

Calculations

Rates of whole-body carbohydrate and fat oxidation were calculated using stoichiometric equations (25). Nitrogen excretion rate was assumed to be $135 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (26). Rates of appearance (R_a) and disappearance (R_d) of glucose at rest were calculated by using the steady-state tracer dilution equation (27). During exercise, glucose R_a and R_d were calculated using the non-steady-state equation of Steele, modified for use with stable isotopes (24). The volume of distribution of glucose was assumed to be 100 ml/kg .

Statistical Analysis

Comparisons between the three trials over time were analyzed by a two-way ANOVA with repeated-measures. The Tukey-Kramer HSD test was used to locate specific differences between means. Statistical significance was set at $\alpha = 0.05$, and all data are presented as mean \pm SE.

Results

Gas Exchange

There were no significant differences detected among treatments C, F, and P, respectively, for VO_2 during 30LT (3.00 ± 0.30 , 3.12 ± 0.28 , and $3.04 \pm 0.27 \text{ L/min}$). Treatment had no effect on whole-body carbohydrate oxidation rate at any time during exercise (30LT or TT; $p > .05$; Table 2). Whole-body fat oxidation rates for the last 5 min of 30LT, and during the TT were higher for F and P when compared to C ($p < .01$; Table 3).

Muscle Glycogen

The dietary treatments had no significant effects on muscle glycogen concentrations before or after 30LT, or glycogen use ($p < .05$; Figure 1). Muscle glycogen results were available for 6 of 8 subjects; one was unwilling to undergo the biopsy procedure, and the biopsy material from another was insufficient for analysis.

Table 2 Carbohydrate Oxidation Rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) After the Consumption of an Isocaloric Preexercise Meal High in Carbohydrate (C), Fat (F), or a Placebo (P) During the First and Last 5 min of 30 min of Exercise Above Lactate Threshold (30LT) and a Subsequent 20-km Time Trial (TT)

Time (min)	C	F	P
0-5	440.8 ± 29.5	393.9 ± 24.2	365.2 ± 19.7
25-30	374.3 ± 23.4	318.3 ± 21.9	311.5 ± 20.4
TT	342.5 ± 34.0	296.4 ± 27.2	282.8 ± 28.7

Note. All values are means \pm SE. There were no statistically significant differences.

Table 3 Fat Oxidation Rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) After the Consumption of an Isocaloric Pre-exercise Meal High in Carbohydrate (C), Fat (F), or a Placebo (P) During the First and Last 5 Min of 30 Min of Exercise Above Lactate Threshold (30LT) and a Subsequent 20-km Time Trial (TT)

Time (min)	C	F	P
0-5	0 ± 0	0 ± 0	$1.6 \pm 1.1^\dagger$
25-30	3.2 ± 1.6	$13.3 \pm 1.6^*$	$15.9 \pm 2.7^*$
TT	4.8 ± 2.1	$16.5 \pm 2.7^*$	$17.0 \pm 3.2^*$

Note. All values are means \pm SE. *F and P > C ($p < .01$); † P > C and F ($p < .01$).

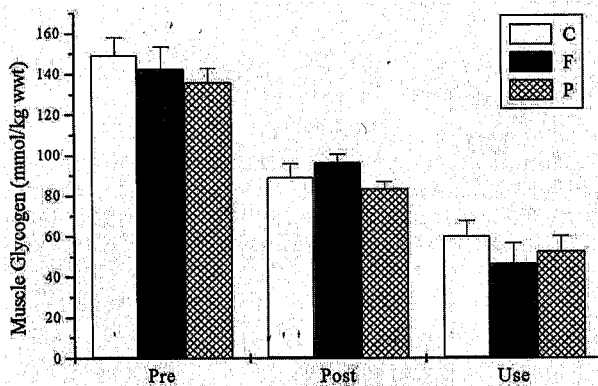


Figure 1 — The effect of the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) on muscle glycogen concentrations before and after 30 min of exercise above lactate threshold (30LT) and muscle glycogen use. All values are means \pm SE. Pre = before 30LT; Post = after 30LT; Use = difference between Pre and Post. There were no differences between treatments

Glucose Kinetics

Glucose R_a (Figure 2) and R_d (Figure 3) were significantly greater for C when compared to F or P only at rest ($p < .01$).

Plasma Metabolites

Plasma glucose concentration was significantly different between treatments ($p < .05$; Figure 4). Plasma glucose concentrations at 120 min before exercise were lower after F and P when compared to C. During 10, 20, and 30 min of 30LT, and immediately prior to the TT, plasma glucose concentrations were higher for F and P when compared to C ($p < .01$). Plasma lactate concentrations were not significantly different

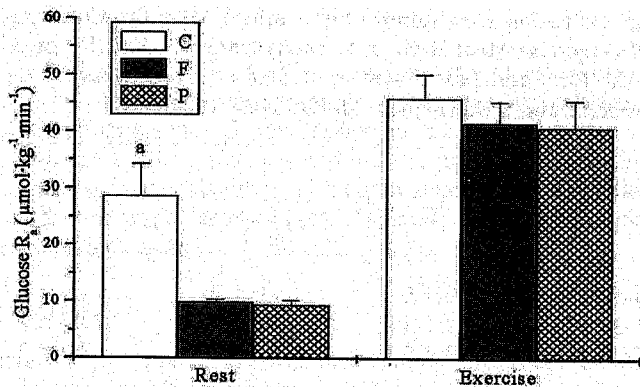


Figure 2 — Plasma glucose rate of appearance (R_a) after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) at rest and during 30 min of exercise above lactate threshold (30LT). All values are means \pm SE. *F and P < C ($p < .01$).

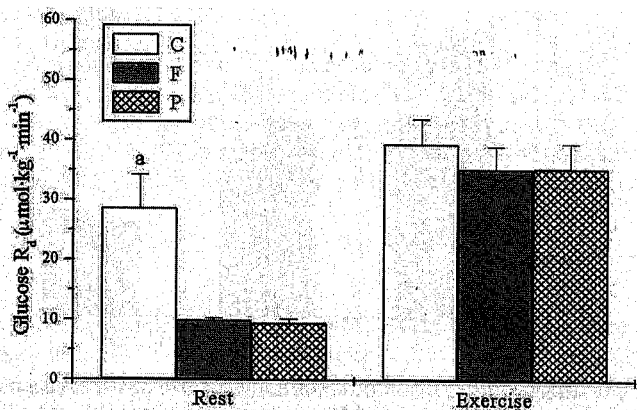


Figure 3 — Plasma glucose rate of disappearance (R_d) after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) at rest and during 30 min of exercise above lactate threshold (30LT). All values are means \pm SE. *F and P < C ($p < .01$).

between treatments (C: 5.0 ± 1.34 , F: 5.64 ± 4.58 , P: 5.0 ± 3.95 mmol/L; $p > .05$). Serum FFA and glycerol concentrations were higher at all time points (30LT and TT) for F and P ($p < .01$) when compared to C, but were not different between F and P (Figures 5 & 6). Serum TG was significantly higher for F when compared to C and P for all time points (30LT and TT) except before the TT (PR-TT; $p < .01$; Figure 7).

Insulin

Serum insulin concentrations were significantly higher for C than for F or P at 0, 10, and 20 min of 30LT ($p < .01$; Figure 8). P was greater than C or F before the TT ($p < .05$).

present study, compared to the aforementioned investigations that were able to produce plasma FFA concentrations of 0.6 to 1.5 mM with the infusion of Intralipid or a fat meal, followed by a heparin injection. Romijn et al. (3) suggested that muscle glycogen use may be reduced if FFA availability is increased to a high concentration (0.6–1.0 mM) from an initial low level (0.2–0.3 mM). Since the peak FFA concentrations in this investigation were approximately 0.4 mM, it is possible that elevat-

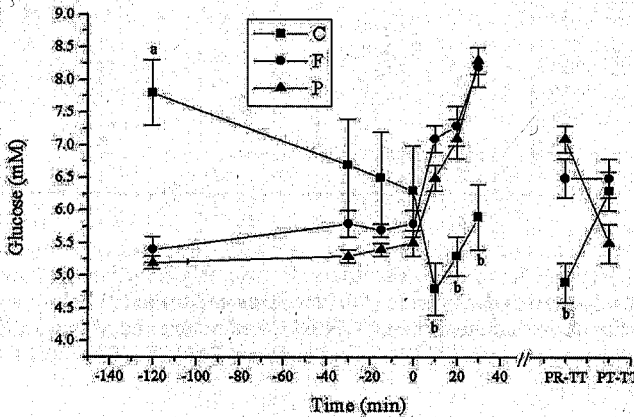


Figure 4 — Plasma glucose concentrations after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) at rest, during 30 min of exercise above lactate threshold (30LT), and before and after a 20-km time trial (TT). All values are means \pm SE. PR-TT = Pre Time Trial; PT-TT = Post Time Trial. *F and P < C ($p < .05$); *F and P > C ($p < .05$).

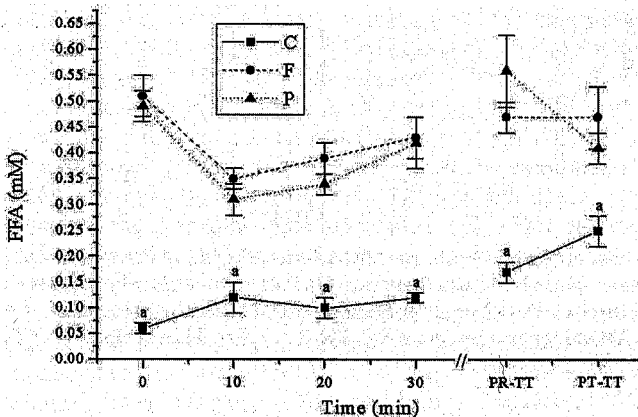


Figure 5 — Serum FFA concentrations after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) before and during 30 min of exercise above lactate threshold (30LT), and before and after a 20-km time trial (TT). All values are means \pm SE. PR-TT = Pre Time Trial; PT-TT = Post Time Trial. *F and P > C ($p < .01$).

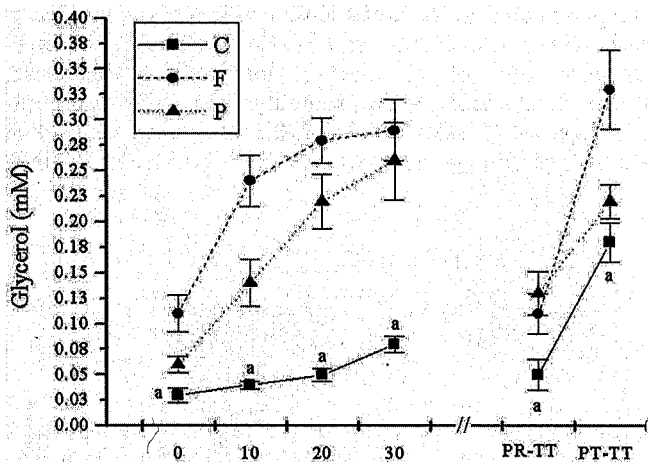


Figure 6 — Serum glycerol concentrations after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) before and during 30 min of exercise above lactate threshold (30LT), and before and after a 20-km time trial (TT). All values are means \pm SE. PR-TT = Pre Time-Trial; PT-TT = Post Time-Trial. ^aF and P > C ($p < .01$).

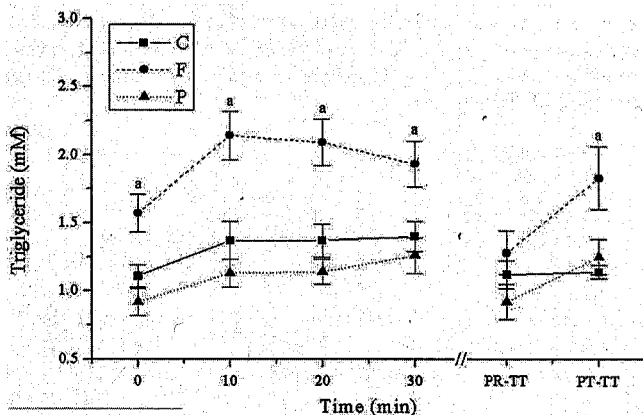


Figure 7 — Serum triglyceride concentrations after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) before and during 30 min of exercise above lactate threshold (30LT), and before and after a 20-km time trial (TT). All values are means \pm SE. PR-TT = Pre Time Trial; PT-TT = Post Time Trial. ^aF > C and P ($p < .01$).

Time Trial Variables

There were no significant differences among treatments for the 20-km time (32.7 ± 0.5 , 33.1 ± 1.1 , and 33.0 ± 0.8 min), power (238 ± 10 , 237 ± 18 , and 235 ± 14 W) or heart rate (163 ± 3 , 166 ± 4 , and 166 ± 4 beats/min) for C, F, and P, respectively.

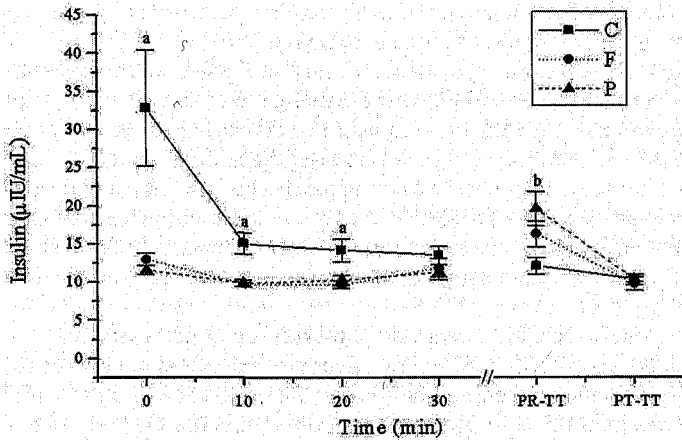


Figure 8 — Serum insulin concentrations after the consumption of an isocaloric pre-exercise meal high in carbohydrate (C), fat (F), or a placebo (P) before and during 30 min of exercise above lactate threshold (30LT), and before and after a 20-km time trial (TT). All values are means \pm SE. PR-TT = Pre Time Trial. PT-TT = Post Time Trial. ^aF and P < C ($p < .01$); ^bP > C and F ($p < .01$).

Discussion

The results of the present investigation are consistent with those of others who found higher concentrations of plasma FFA after a pre-exercise fat meal when compared to a carbohydrate meal (28–30). Despite the differences in FFA concentrations at an exercise intensity associated with impaired availability of FFA, whole-body fat and carbohydrate oxidation, muscle glycogen utilization, and glucose flux were not greatly altered. Last, there were no differences in time trial performance.

The increased rate of whole-body fat oxidation after F and P was insufficient to demonstrate a “sparing” of muscle glycogen when compared to C. Although the differences in whole-body fat oxidation between C and F/P were statistically significant, the estimated reduction in fat use during 30LT and TT was only ≈ 7 g. In turn, the estimated sparing of carbohydrate for F/P when compared to C was ≈ 33 g for whole-body carbohydrate oxidation and ≈ 5 g for blood glucose (based on glucose R_d). Therefore, the estimated reduction in muscle glycogen utilization was ≈ 28 g of carbohydrate, which was too small to produce detectable differences in muscle glycogen concentration. These results support the study by Burke et al. (31), who also used a combination of respiratory gas measurements, glucose tracer, and biopsy techniques to study changes in endogenous carbohydrate utilization. These investigators calculated a sparing of ≈ 70 g of carbohydrate between treatments (fat-adapted and high carbohydrate feedings) during 120 min of exercise at 70% $\dot{V}O_{2max}$, yet were also unable to detect a statistically significant difference in muscle glycogen use.

This lack of a muscle glycogen sparing effect is in contrast to several studies that found a reduced rate of muscle glycogen utilization when plasma FFA concentrations were elevated (3–5). This discrepancy may be explained by the relatively small increases in FFA concentrations (0.4 mM) resulting from F and P in the

ing FFA to a concentration that produces a carbohydrate-sparing effect (>0.7 mM) requires a much longer fast (32) or a heparin injection.

The lack of a significant effect of meal composition on substrate oxidation after pre-exercise meals in the present investigation supports those of others (11–13). However, Hawley et al. (6) demonstrated decreased whole-body carbohydrate and increased whole-body fat oxidation after the administration of a fat meal followed by a heparin injection when compared to a carbohydrate meal and a carbohydrate meal with nicotinic acid ($80\% \text{VO}_{2\text{max}}$). It is difficult to explain the divergent results, but one explanation may be related to the fact that the FFA concentrations of Hawley et al. (6) were much higher than the present and aforementioned investigations.

The present results also provide no evidence of decreased use of blood glucose during exercise (therefore liver glycogen stores), since the glucose R_d was lower only after the fat meal and fast during the pre-exercise period. This lack of an effect of elevated FFA concentrations on blood glucose R_d is consistent with the results of Romijn et al. (3) who used tracer techniques and Odland et al. (33) who used the arterio-venous balance technique.

The results of this investigation do not rule out the notion that increasing the availability of FFA will reduce carbohydrate oxidation during high intensity exercise, as indicated by several other investigations (3–6). The results of the TG analysis (Figure 7) indicate that the FFA from F were present in the blood stream before and during exercise. However, the lack of an increase in FFA concentrations after F when compared to P (Figure 5) suggest that little or none of the exogenously-supplied FFA were present as non-esterified fatty acids (NEFA) in the blood, thus not bound to albumin or readily available for oxidation by the exercising muscle. The exogenously-supplied FFA from F were likely unable to be “liberated” from lipoproteins and, in turn, contribute significantly to substrate oxidation. Based on the chain lengths of the FFA contained in F (16:0, 18:0, and 18:1), the predominant carrier of those FFA would be chylomicrons (18). There is little evidence that FFA bound to lipoproteins are a significant source of energy during exercise (34). Heparin injections could increase lipoprotein lipase (LPL) activity 100–300 times and liberate the exogenously supplied FFA from the lipoproteins (35). However, heparinized blood samples represent the highest potential of LPL lipolysis, not necessarily what occurs under ordinary circumstances (35). Thus, it is possible that the activity of LPL without heparin is insufficient to increase plasma NEFA concentrations to the level necessary to produce a carbohydrate sparing effect, irrespective of the amount of fat consumed in a meal.

TT performance was not different between the treatments in this study, which supports Hawley et al. (6) but is contrasted by others (11–13). Since the explanation for fatigue during this type of exercise is not well understood, it is difficult to explain the differences in time trial performance between these studies. One explanation for a lack of an effect of pre-exercise meal on performance may be that muscle glycogen depletion was not the cause of fatigue. The subjects entered TT with muscle glycogen concentrations of approximately 90 mmol/kg wwt. Assuming a uniform rate of muscle glycogen utilization during TT, the projected muscle glycogen concentrations at the end of the TT would be approximately 37 mmol/kg wwt at the end of the TT. This value is very similar to the muscle glycogen concentrations found at fatigue in the study by Coyle et al. (36) (37–39 mmol/kg wwt). However, since the rate of muscle glycogen utilization decreases as exercise duration increases (37) and

that the carbohydrate oxidation rates during the first 5 min of TT were lower than the last 5 min of 30LT, it is likely that the post exercise muscle glycogen concentrations were higher than those reported by Coyle et al. (36). Pre- and post-TT blood glucose concentrations were 6.1 and 6.2 mM, respectively, so the subjects were not hypoglycemic at the beginning or the end of the TT. Thus, it appears that blood glucose concentrations may have been sufficient to compensate for lowered muscle glycogen concentrations (36).

An alternative explanation for why some studies fail to detect an improvement in performance with carbohydrate supplementation may be related to subject selection and study design. Clark et al. (17) compared the effect of carbohydrate ingestion to the mere suggestion that subjects received carbohydrate (placebo effect) during 40-km time trial performance. The results of the study suggest that mere knowledge of treatment (carbohydrate supplementation) can improve performance, and implementation of familiarization trials can reduce variability in performance (particularly in sub-elite competitors). To circumvent the potential confounding placebo effects of a meal, the present investigation utilized liquid meals of indistinguishable taste and texture. The subjects in the present investigation were competitive cyclists or triathletes, and performed at least one familiarization trial prior to the formal experimental sessions. We also utilized a performance test (measured time required to complete a fixed amount of work) that has been shown to be reliable and valid (21), particularly when compared to tests that are terminated by exhaustion of the subject. If the assumptions of Clark et al. (17) are correct, some of the differences between the present study (and Hawley et al., 6) and others (11, 12) may be related to a placebo effect, subject selection, and familiarization trials. However, the differences between El Sayed et al. (13) and the present investigation, cannot be explained by these factors.

In conclusion, the results of the present study indicate that the macronutrient composition of a meal prior to high intensity endurance exercise elicits minor effects on whole-body fat oxidation, and no effect on whole-body carbohydrate oxidation, glucose kinetics, or muscle glycogen use when compared to a fast. In turn, there is no evidence to suggest that pre-exercise meal composition has any effect on subsequent 20-km time trial performance. Assuming that pre-exercise carbohydrate stores are not compromised, it appears as though a meal may not be necessary prior to intense exercise that lasts approximately 1 h. It is likely that a pre-exercise meal alone cannot produce physiologically significant increases in plasma FFA unless it is followed by a heparin injection. Heparin injections, however, are not practical or ethical in athletic competition.

References

1. Brooks, G.A., and J. Mercier. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J. Appl. Physiol.* 76:2253-2261, 1994.
2. Romijn, J.A., E.F. Coyle, S. Sidossis, A. Gastaldelli, J.F. Horowitz, E. Endert, and R.R. Wolfe. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* 265: (Endocrinol. Metab. 28:) E380-E391, 1993.
3. Romijn, J.A., E.F. Coyle, L.S. Sidossis, X.-J. Zhang, and R.R. Wolfe. Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. *J. Appl. Physiol.* 79:1939-1945, 1995.

4. Dyck, D.J., C.T. Putman, G.J.F. Heigenhauser, E. Hultman, and L.L. Spriet. Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. *Am. J. Physiol.* 265: (Endocrinol. Metab. 28:) E852-E859, 1993.
5. Dyck, D.J., S.J. Peters, P.S. Wendling, A. Chesley, E. Hultman, and L.L. Spriet. Regulation of muscle glycogen phosphorylase activity during intense aerobic cycling with elevated FFA. *Am. J. Physiol.* 270: (Endocrinol. Metab. 33:) E116-E125, 1996.
6. Hawley, J.A., L.M. Burke, D.J. Angus, K.E. Fallon, D.T. Martin, and M.A. Febbraio. Effect of altering substrate availability on metabolism and performance during intense exercise. *Br. J. Nutr.* 84:829-838, 2000.
7. Bergstrom, J., L. Hermansen, E. Hultman, and B. Saltin. Diet, muscle glycogen and physical performance. *Acta. Physiol. Scand.* 71:140-150, 1967.
8. Hermansen, L., E. Hultman, and B. Saltin. Muscle glycogen during prolonged severe exercise. *Acta. Physiol. Scand.* 71:129-139, 1967.
9. Coggan, A.R., and E.F. Coyle. Effect of carbohydrate feedings during high-intensity exercise. *J. Appl. Physiol.* 65:1703-1709, 1988.
10. Hawley, J.A., E.J. Schabort, T.D. Noakes, and S.C. Dennis. Carbohydrate-loading and exercise performance: an update. *Sports Med.* 24:73-81, 1997.
11. Foster, C., D.L. Costill, and W.J. Fink. Effects of preexercise feedings on endurance performance. *Med. Sci. Sports Exerc.* 11:1-5, 1979.
12. Nuefer, P.D., D.L. Costill, M.G. Flynn, J.P. Kirwan, J.B. Mithcell, and J. Houmard. Improvements in exercise performance: effects of carbohydrate feedings and diet. *J. Appl. Physiol.* 62:983-988, 1987.
13. El-Sayed, M.S., J. Balmer, and A.J.M. Rattu. Carbohydrate ingestion improves endurance performance during a 1 h simulated cycling time trial. *J. Sport Sci.* 15:223-230, 1997.
14. Coyle, E.F., M.E. Feltner, S.A. Kautz, M.T. Hamilton, S.J. Montain, L.D. Baylor, and G.W. Petrek. Physiological and biomechanical factors associated with elite endurance cycling performance. *Med. Sci. Sports Exerc.* 23:93-107, 1991.
15. Wilmore, J.H. A simplified method for determination of residual lung volumes. *J. Appl. Physiol.* 27:96-100, 1969.
16. Siri, W.E. Body composition from fluid spaces and density. In: *Techniques for measuring Body Composition*, J. Brozek and A. Henschel (Eds.). Washington, DC: National Academy of Sciences, 1961, pp. 223-244.
17. Clark, V.R., W.G. Hopkins, J.A. Hawley, and L.M. Burke. Placebo effect of carbohydrate feedings during a 40-km cycling time trial. *Med. Sci. Sports Exerc.* 32:1642-1647, 2000.
18. Rhee, S.K., A.J. Kayani, A. Cizek, and J.T. Brenna. Desaturation and interconversion of dietary stearic and palmitic acids in human plasma and lipoproteins. *Am. J. Clin. Nutr.* 65:451-458, 1997.
19. Mora-Rodriguez, R., and E.F. Coyle. Effects of plasma epinephrine on fat metabolism during exercise: interactions with exercise intensity. *Am. J. Physiol. Endocrinol. Metab.* 278:E669-E676, 2000.
20. Coggan, A.R., W.M. Kohrt, R.J. Spina, J.P. Kirwan, D.M. Bier, and J.O. Holloszy. Plasma glucose kinetics during exercise in subjects with high and low lactate thresholds. *J. Appl. Physiol.* 73:1873-1880, 1992.
21. Jeukendrup, A., W.H.M. Saris, F. Brouns, and A.D.M. Kester. A new validated endurance performance test. *Med. Sci. Sports Exerc.* 28:266-270, 1996.
22. Passonneau, J.V., and V.R. Lauderale. A comparison of three methods of glycogen measurement in tissues. *Anal. Biochem.* 60:405-412, 1974.

23. Geor, R.J., K.W. Hinchcliff, and R.A. Sams. Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise. *J. Appl. Physiol.* 88:1765-1776, 2000.
24. Wolfe, R.R. *Radioactive and Stable Isotope Tracers in Biomedicine*. New York: Wiley-Liss, 1992.
25. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* 55:628-634, 1983.
26. Carraro, F., C.A. Stuart, W.H. Hartl, J. Rosenblatt, and R.R. Wolfe. Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 259: (Endocrinol. Metab. 22:) E470-E476, 1990.
27. Steele, R. Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann. NY. Acad. Sci.* 82:420-430, 1959.
28. Okano, G., Y. Sato, Y. Takumi, and M. Sugawara. Effect of 4 h preexercise high carbohydrate and high fat meal ingestion on endurance performance and metabolism. *Int. J. Sports. Med.* 17:530-534, 1996.
29. Okano, G., Y. Sato, and Y. Murata. Effect of elevated blood FFA levels on endurance performance after a single fat meal ingestion. *Med. Sci. Sports. Exerc.* 30:763-768, 1998.
30. Whitley, H.A., S.M. Humphreys, I.T. Campbell, M.A. Keegan, T.D. Jayanetti, D.A. Sperry, D.P. MacLaren, T. Reilly, and K.N. Frayn. Metabolic and performance responses during endurance exercise after high-fat and high-carbohydrate meals. *J. Appl. Physiol.* 85:418-424, 1998.
31. Burke, L.M., D.J. Angus, G.R. Cox, N.K. Cummings, M.A. Febbraio, K. Gawthorn, J.A. Hawley, M. Minehan, D.T. Martin, and M. Hargreaves. Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *J. Appl. Physiol.* 89:2413-2421, 2000.
32. Zinker, B.A., K. Britz, and G.A. Brooks. Effects of a 36-hour fast on human endurance and substrate utilization. *J. Appl. Physiol.* 69:1849-1855, 1990.
33. Odland, M.L., G.J.F. Helgenhauser, D. Wong, M.G. Hollidge-Horvat, and L.L. Spriet. Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. *Am. J. Physiol.* 274: (Regulatory. Integrative. Comp. Physiol. 43:) R894-R902, 1998.
34. Horowitz, J.F., and S. Klein. Lipid metabolism during endurance exercise. *Am. J. Clin. Nutr.* 72(Suppl.):558S-563S, 2000.
35. Pruneta, V., D. Autran, G. Ponsin, C. Marcais, L. Duvillard, B. Verges, F. Berthezene, and P. Moulin. Ex vivo measurement of lipoprotein lipase-dependent very low density lipoprotein (VLDL)-triglyceride hydrolysis in human VLDL: an alternative to the postheparin assay of lipoprotein lipase activity. *J. Clin. Endocrinol. Metab.* 86:797-803, 2001.
36. Coyle, E.F., A.R. Coggan, M.K. Hemmert, and J.L. Ivy. Muscle glycogen during prolonged strenuous exercise when fed carbohydrate. *J. Appl. Physiol.* 61:165-172, 1986.
37. Weltan, S.M., A.N. Bosch, S.C. Dennis, and T.D. Noakes. Influence of muscle glycogen content on metabolic regulation. *Am. J. Physiol.* 274: (Endocrinol. Metab. 37:) E72-E82, 1998.

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